

# Pyrenosetins A–C, New Decalinoylspirotetramic Acid Derivatives Isolated by Bioactivity-Based Molecular Networking from the Seaweed-Derived Fungus *Pyrenochaetopsis* sp. FVE-001

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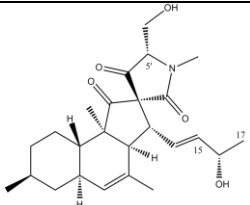
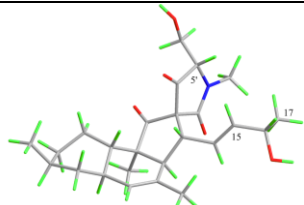
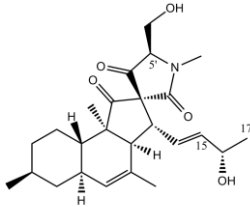
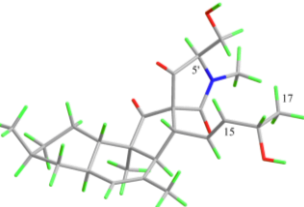
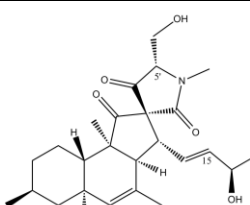
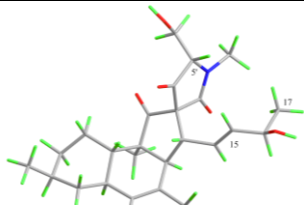
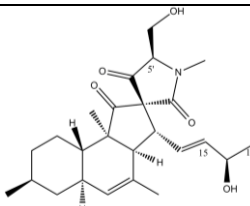
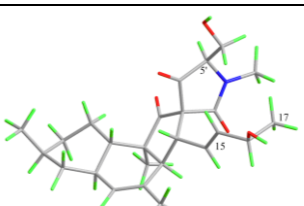
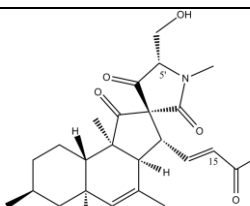
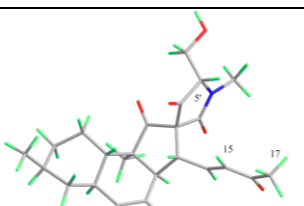
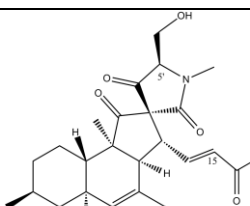
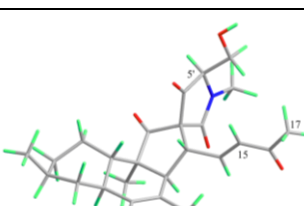
**Table S1.** In vitro anticancer activity (%) of Kupchan subextracts (KH, KC, KM) and SPE fractions against cancer cell lines (A-375, A-549, HT-29, HCT-116, MB-231) and non-cancerous HaCaT cell line.

Cell growth inhibition (%)						
	A-375 (100 $\mu$ g/ml)	A-549 (100 $\mu$ g/ml)	HT-29 (100 $\mu$ g/ml)	HCT-116 (100 $\mu$ g/ml)	MB-231 (100 $\mu$ g/ml)	HaCaT (100 $\mu$ g/ml)
KH	31	0	29	0	0	31
KC	98	99	99	76	99	66
KM	0	0	0	0	0	0
KC Fr.0	0	0	0	0	0	0
KC Fr.1	0	0	0	0	0	0
KC Fr.2	0	0	0	0	0	0
KC Fr.3	0	0	0	0	0	0
KC Fr.4	0	0	0	0	0	0
KC Fr.5	85	54	99	0	63	44
KC Fr.6	99	99	99	99	99	99
KC Fr.7	99	99	99	99	99	99
KC Fr.8	43	0	0	0	0	0
KC Fr.9	0	0	0	0	0	0
KC Fr.10	0	0	0	0	0	0

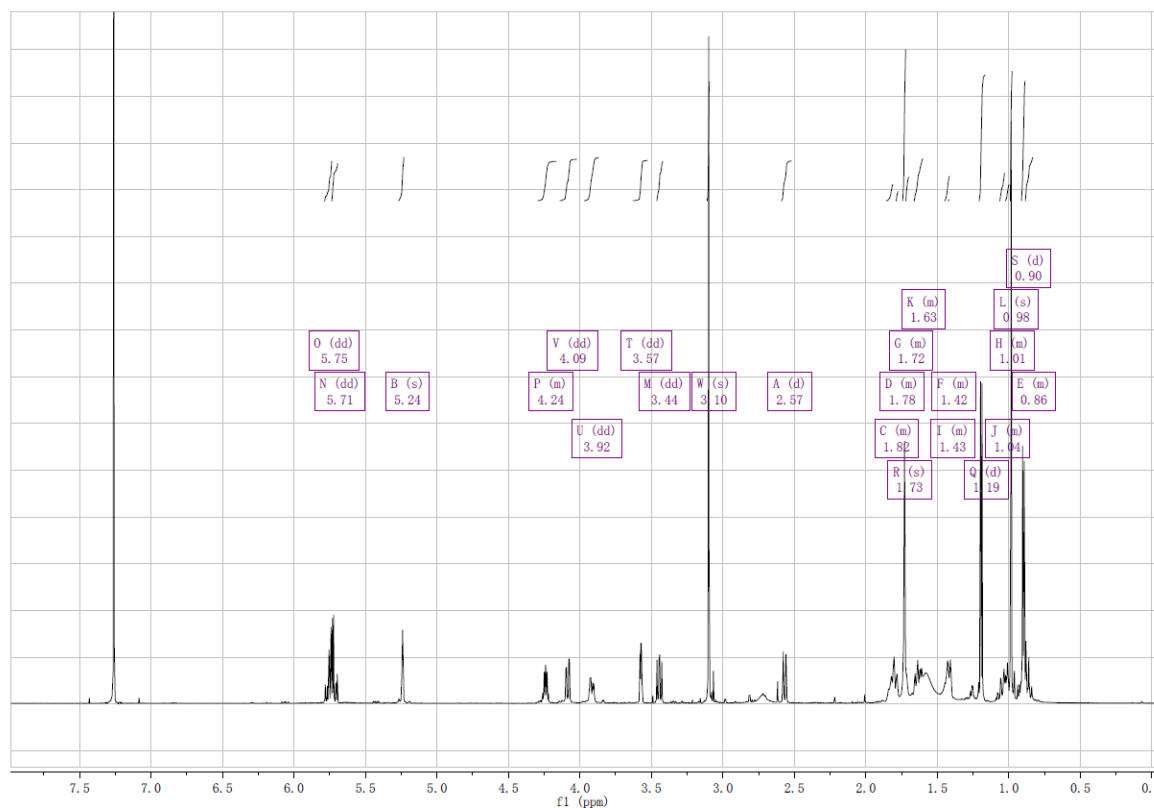
**Table S2.** The  $\Delta\delta(\delta_S-\delta_R)$  data for the *S*- and *R*-MTPA esters **6–9** in  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz).

	<b>6 (S)</b>	<b>7 (R)</b>	$\Delta\delta_{S-R}$	<b>8 (S)</b>	<b>9 (R)</b>	$\Delta\delta_{S-R}$
<b>C</b>	$\delta_{\text{H}}$ , mult ( <i>J</i> in Hz)	$\delta_{\text{H}}$ , mult ( <i>J</i> in Hz)		$\delta_{\text{H}}$ , mult ( <i>J</i> in Hz)	$\delta_{\text{H}}$ , mult ( <i>J</i> in Hz)	
13	3.39, m	3.35, dd (11.5, 9.5)	0.04	3.27, dd (11.3, 9.7)	3.28, dd (11.4, 9.4)	-0.01
14	5.92, dd (15.5, 9.5)	5.84, dd (15.4, 9.8)	0.08	6.09, dd (14.8, 9.7)	6.10, dd (15.3, 9.7)	-0.01
15	5.65, dd (15.5, 6.4)	5.58, dd (15.4, 6.4)	0.07	5.39, dd (14.9, 7.8)	5.52, dd (15.3, 7.6)	-0.13
16	5.46, m	5.45, m	0.01	5.34, m	5.38, m	-0.04
17	1.22, d (6.5)	1.30, d (6.5)	-0.08	1.31, d (6.3)	1.27, d (6.5)	0.04

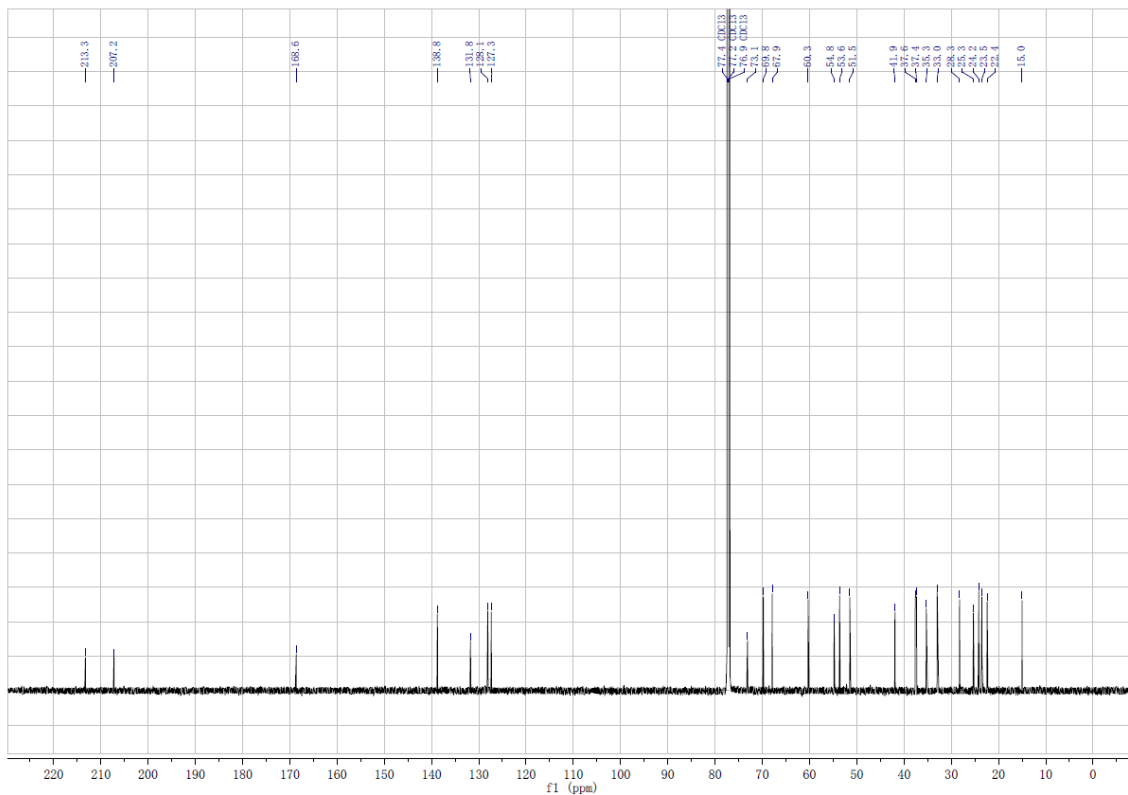
**Table S3.** The distance (Å) between protons H-5', H-15 and H<sub>3</sub>-17 in the tetramic acid portion of the compounds 1-3. The red marking indicates the assigned relative stereochemistry based on measured the distances allowing observable NOE correlations (up to 4 Å) between relevant protons.

Compd	2D Structure	3D model	H-5' orientation	Distance H-5'/H-15	NOE H-5'/H-15	Distance H-5'/H <sub>3</sub> -17	NOE H-5'/H <sub>3</sub> -17
1 (5'-β)			β	2.96 Å	YES	3.28 Å	YES
1 (5'-α)			α	4.83 Å		5.07 Å	
2 (5'-β)			β	2.88 Å		3.33 Å	
2 (5'-α)			α	5.14 Å	NO	6.84 Å	NO
3 (5'-β)			β	3.80 Å		5.11 Å	
3 (5'-α)			α	5.41 Å	NO	6.77 Å	NO

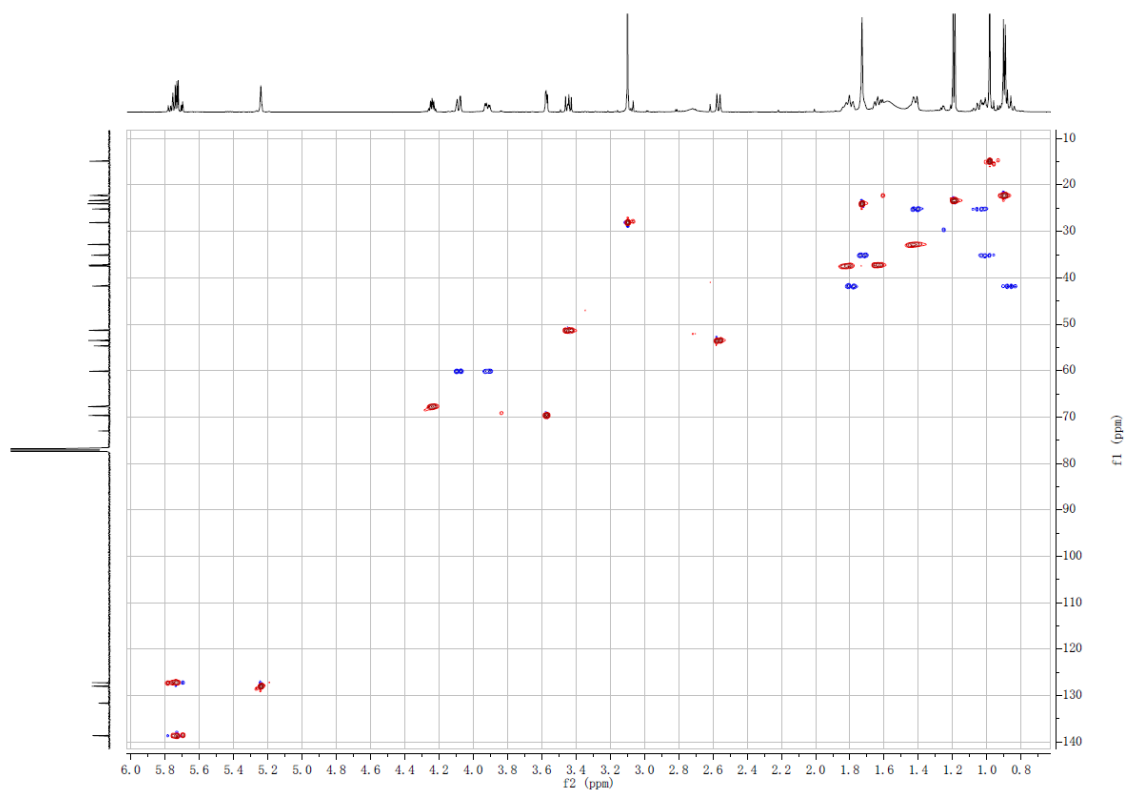
**Figure S1.**  $^1\text{H}$  NMR spectrum of compound **1** (600 MHz,  $\text{CDCl}_3$ ).



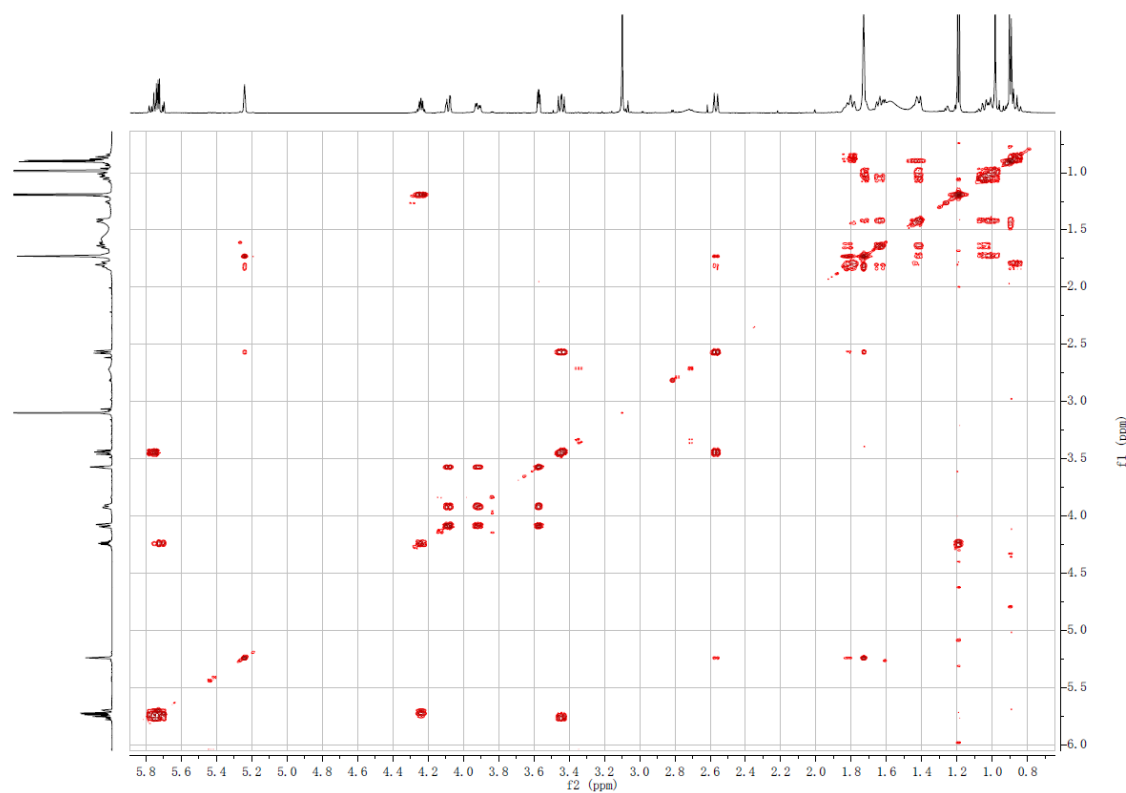
**Figure S2.**  $^{13}\text{C}$  NMR spectrum of compound **1** (150 MHz,  $\text{CDCl}_3$ ).



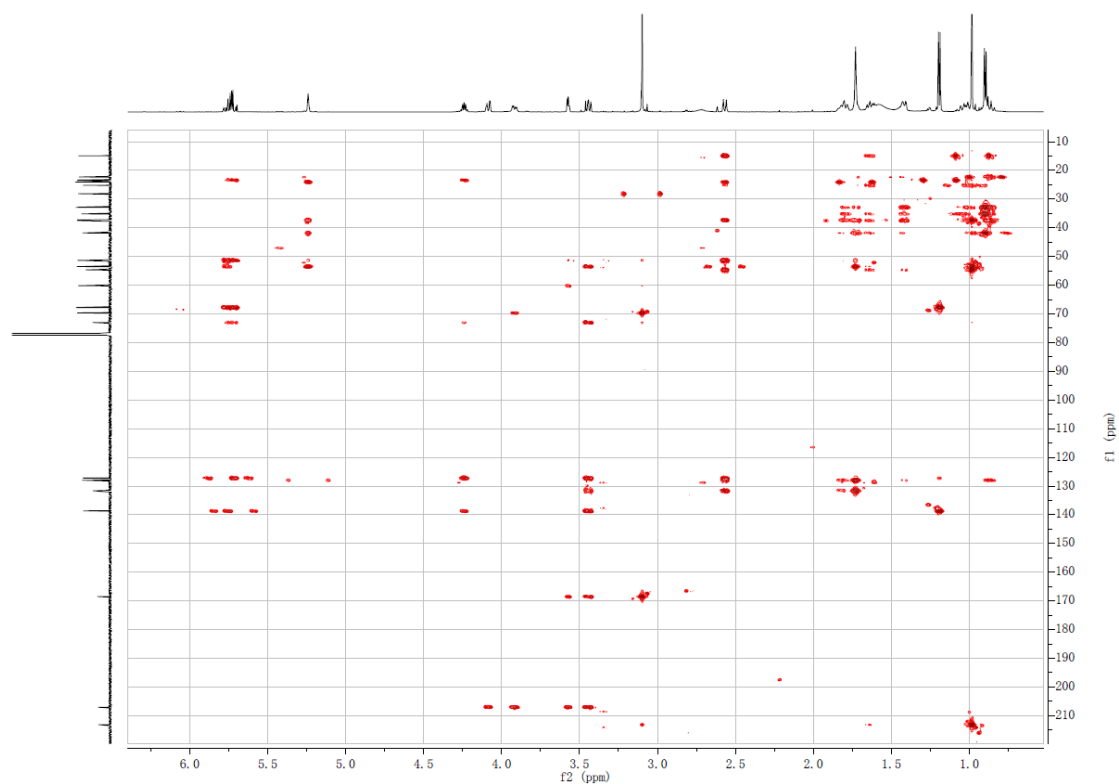
**Figure S3.** DEPT-HSQC spectrum of compound **1** (600 MHz, CDCl<sub>3</sub>).



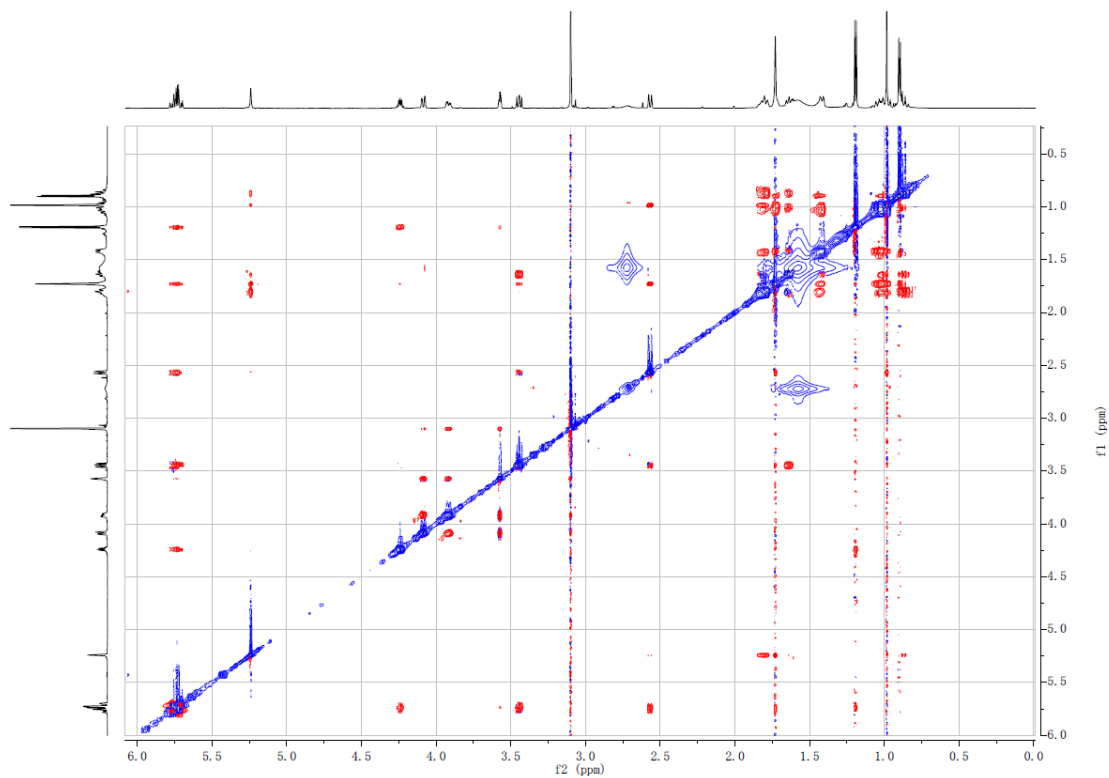
**Figure S4.** COSY spectrum of compound **1** (600 MHz, CDCl<sub>3</sub>).



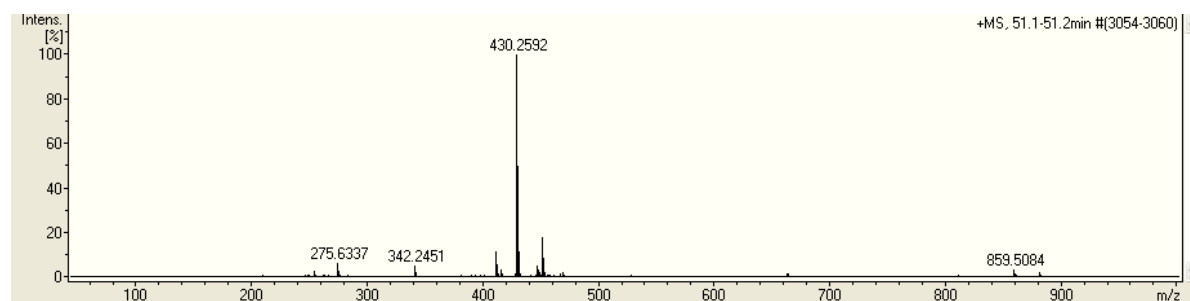
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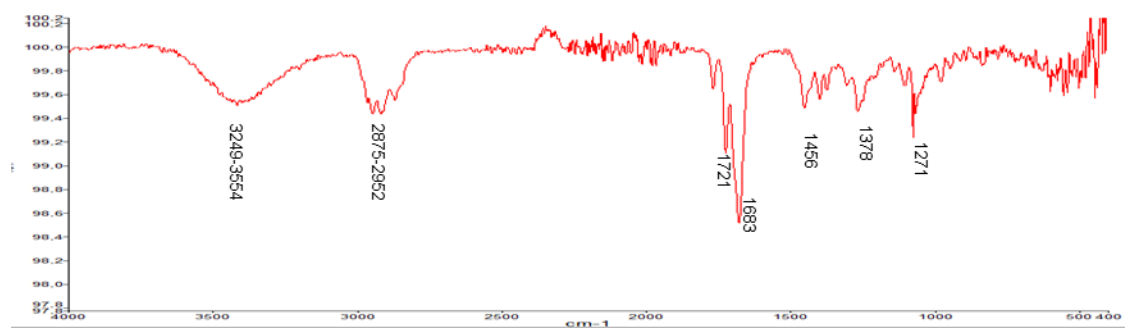
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**Figure S7.** HR-ESIMS spectrum of compound 1.

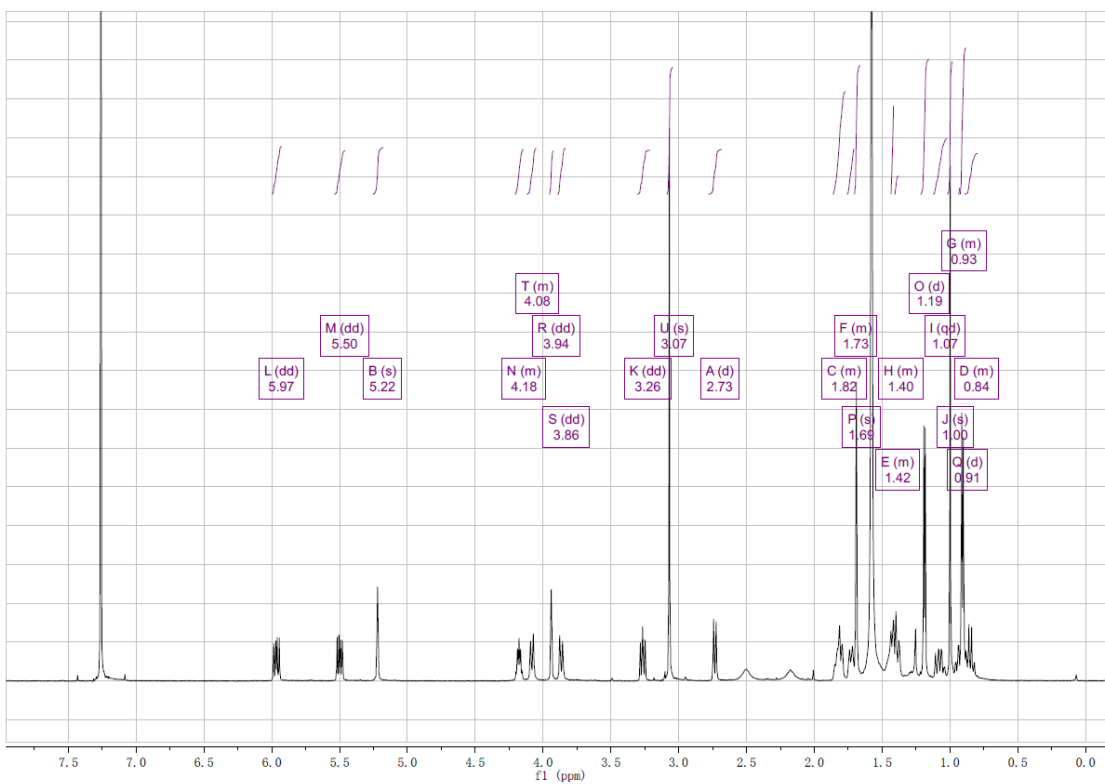


**Figure S8.** FT-IR spectrum of compound 1.





**Figure S9.**  $^1\text{H}$  NMR spectrum of compound **2** (600 MHz,  $\text{CDCl}_3$ ).



**Figure S10.**  $^{13}\text{C}$  NMR spectrum of compound **2** (150 MHz,  $\text{CDCl}_3$ ).

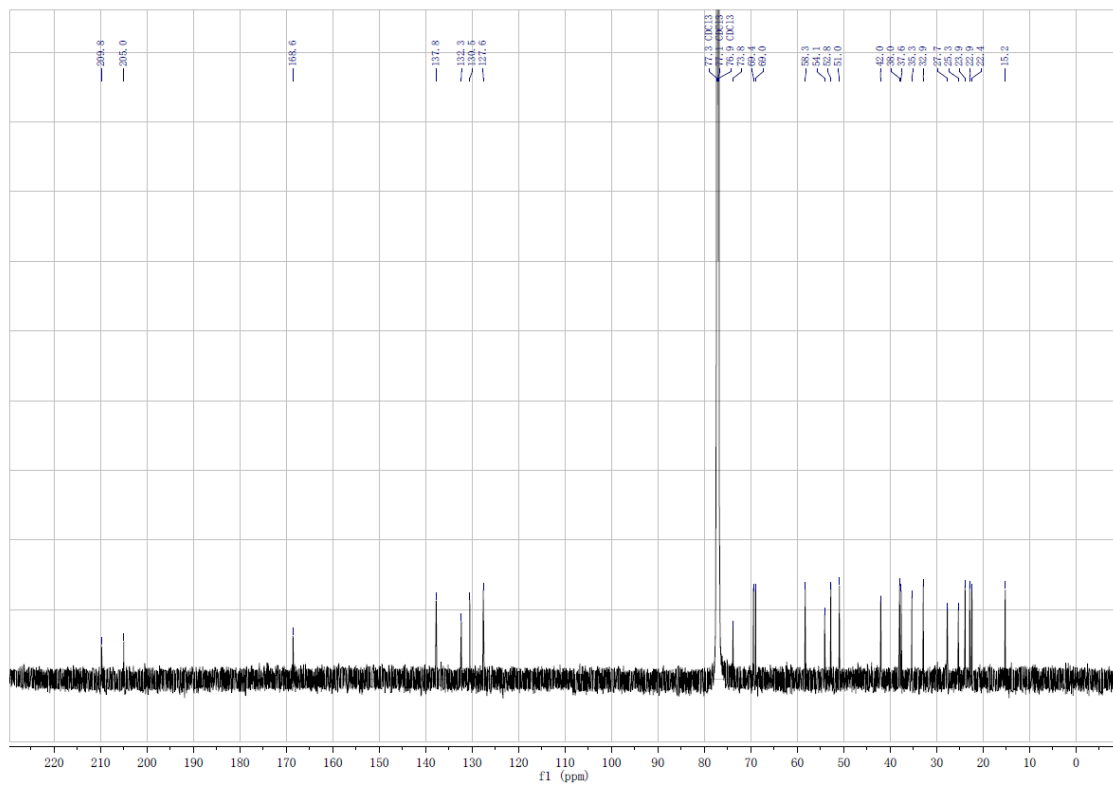


Figure S11. DEPT-HSQC spectrum of compound **2** (600 MHz, CDCl<sub>3</sub>).

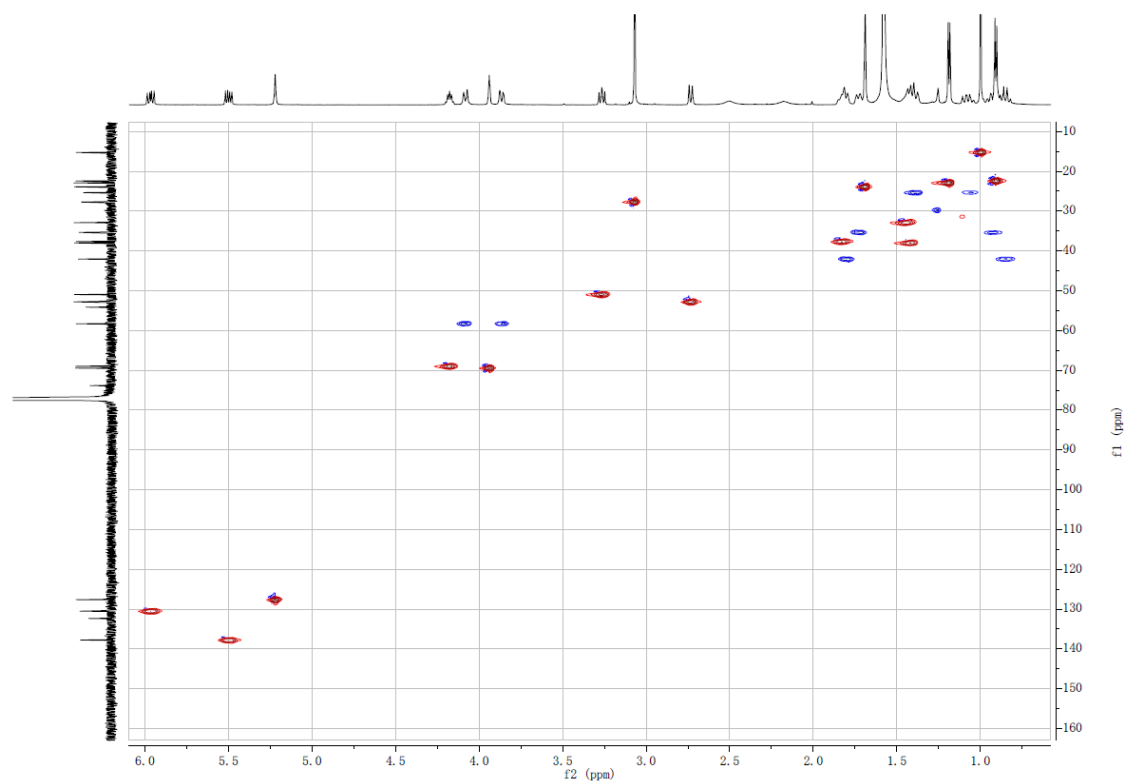


Figure S12. COSY spectrum of compound **2** (600 MHz, CDCl<sub>3</sub>).

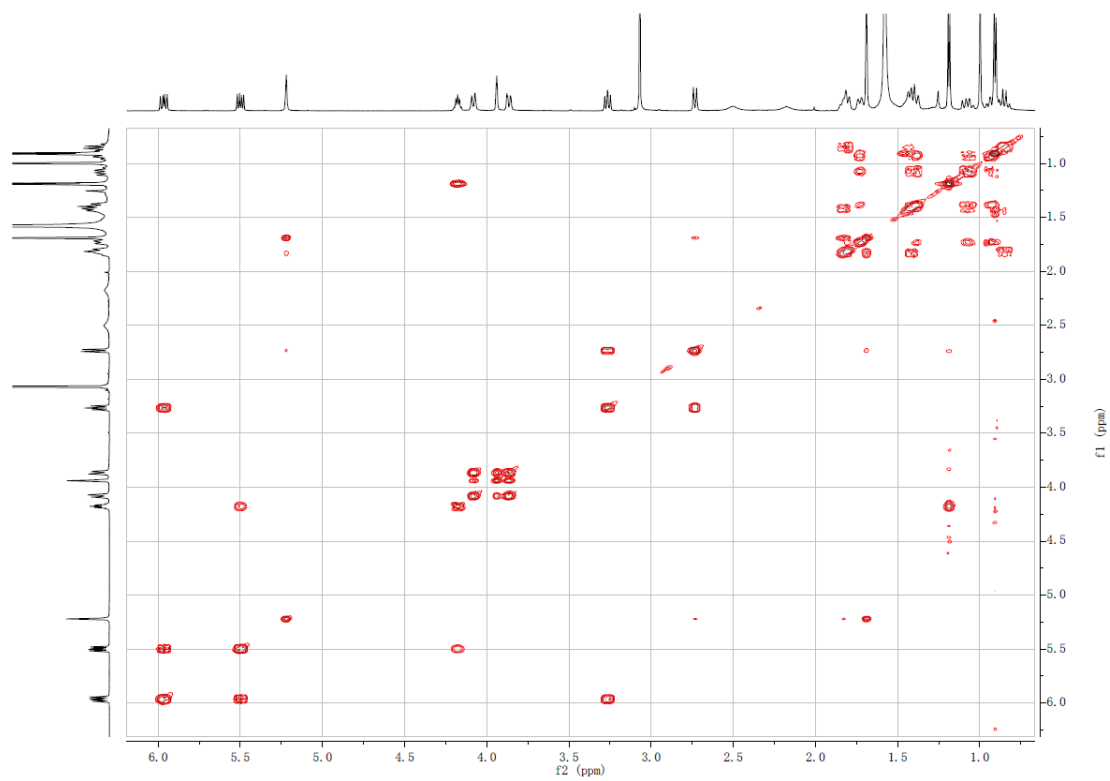


Figure S13. HMBC spectrum of compound **2** (600 MHz, CDCl<sub>3</sub>).

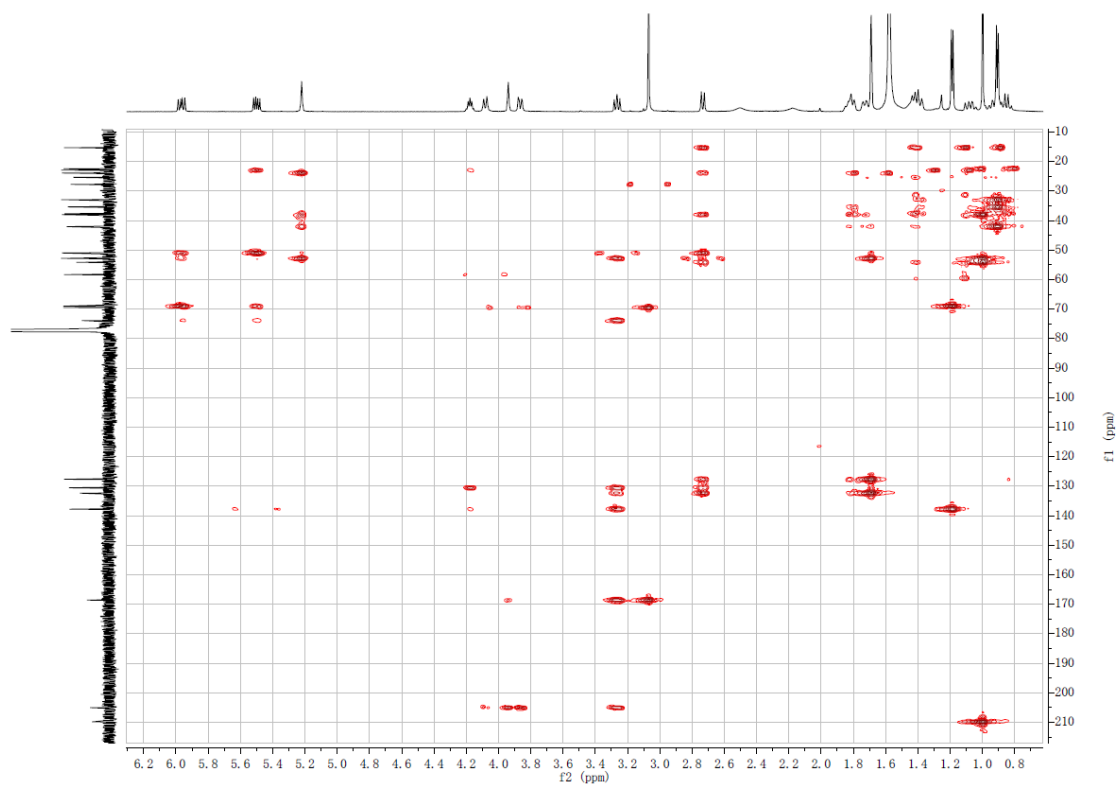
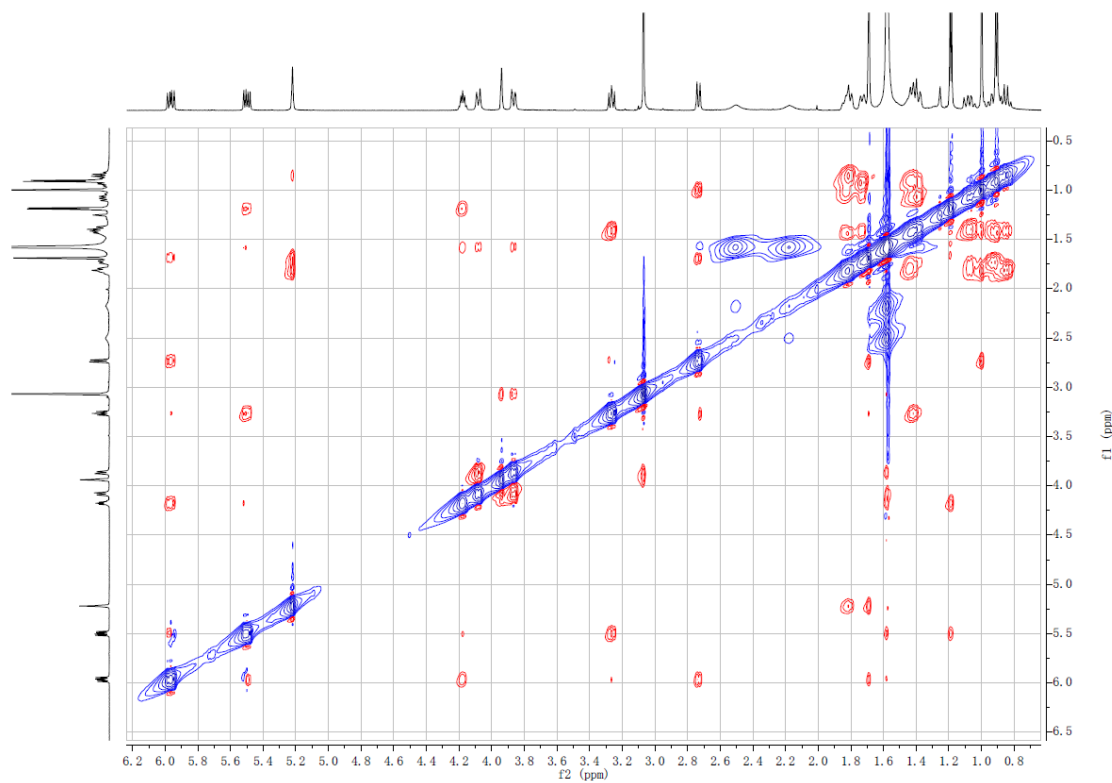
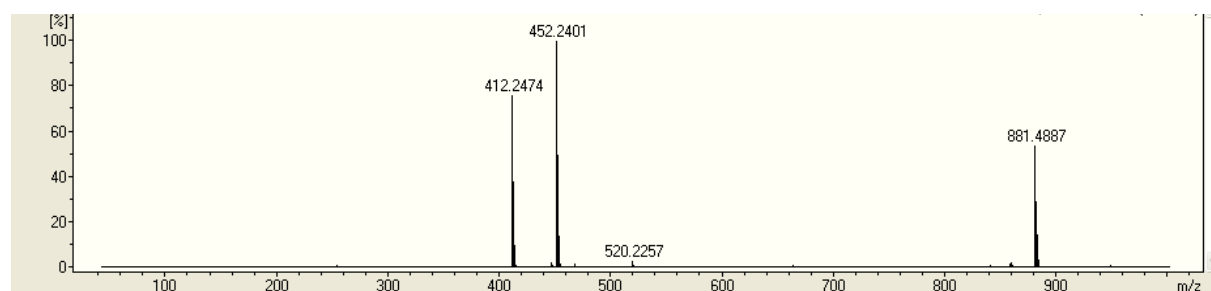


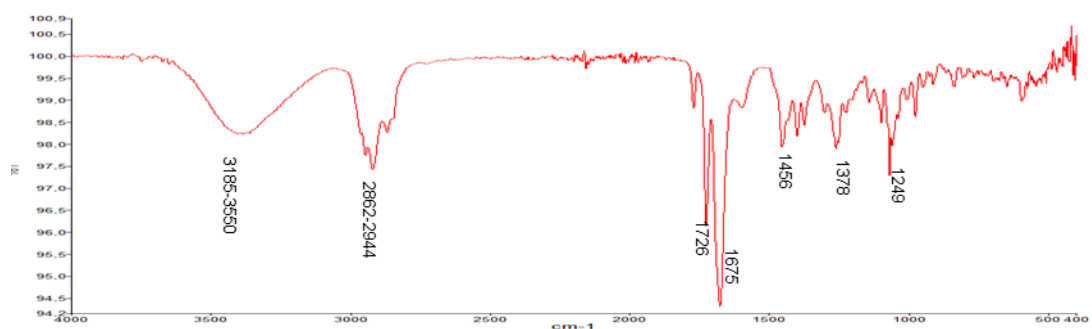
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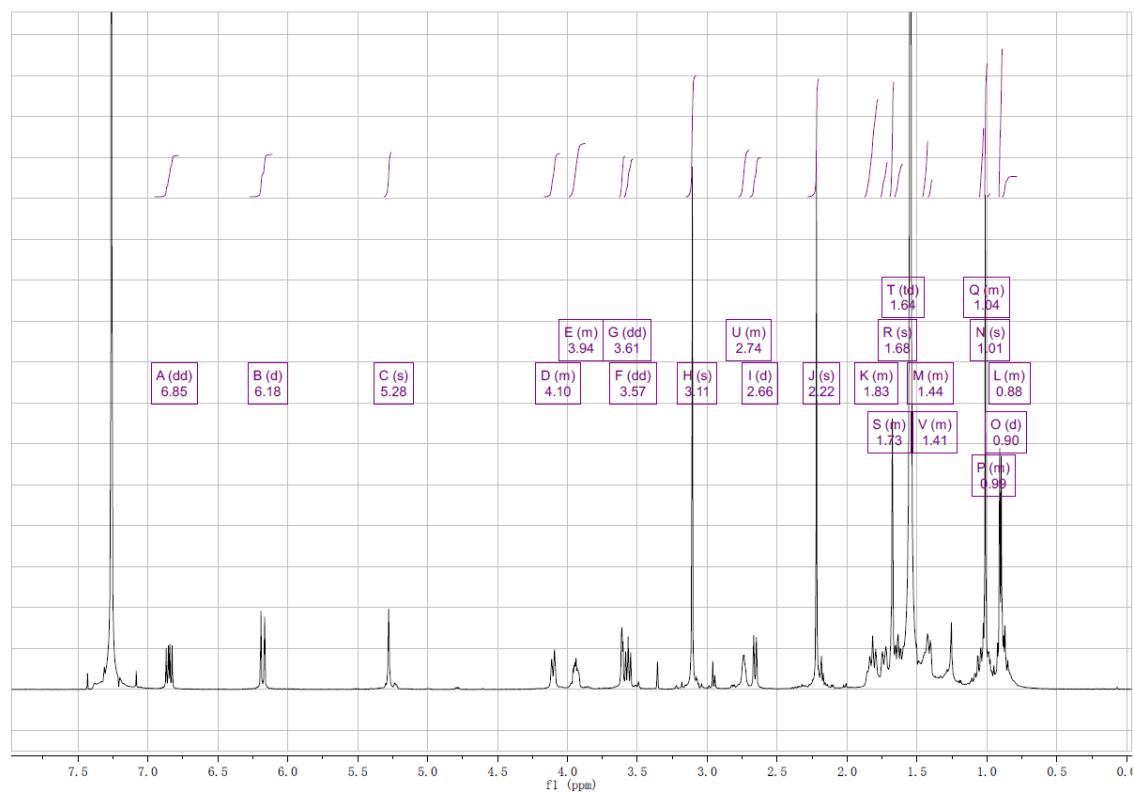
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**Figure S16.** FT-IR spectrum of compound **2**.



**Figure S17.**  $^1\text{H}$  NMR spectrum of compound **3** (600 MHz,  $\text{CDCl}_3$ ).



**Figure S18.**  $^{13}\text{C}$  NMR spectrum of compound **3** (150 MHz,  $\text{CDCl}_3$ ).

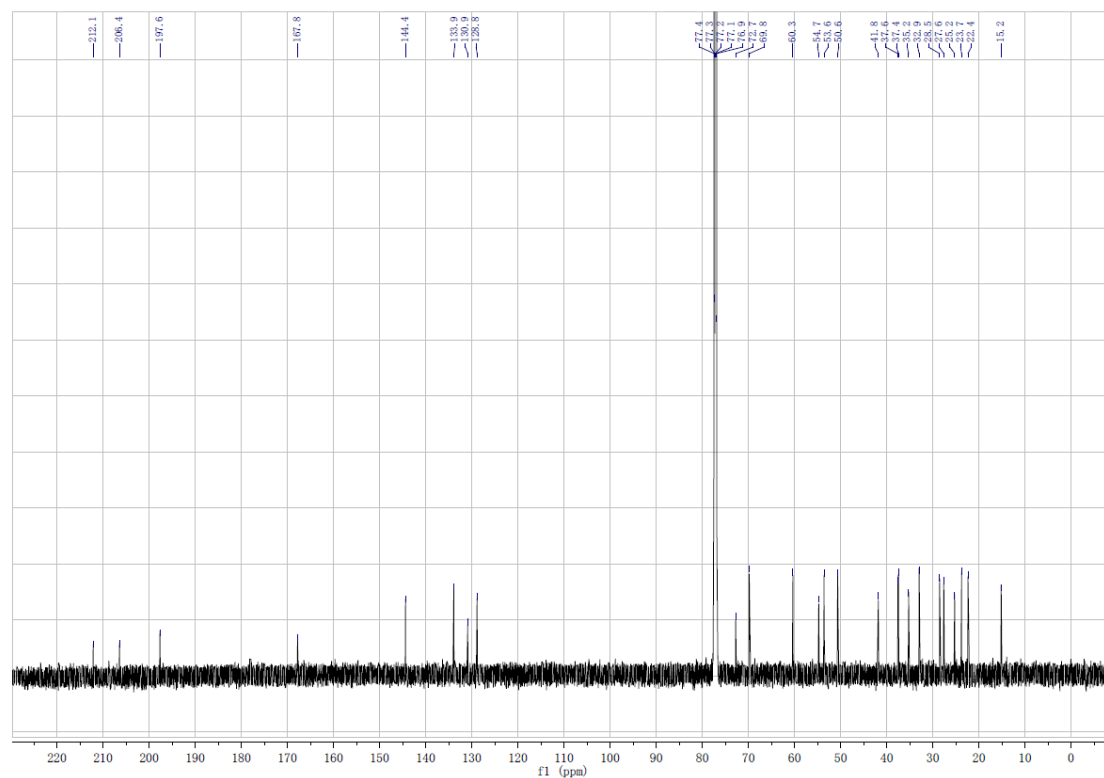


Figure S19. DEPT-HSQC spectrum of compound **3** (600 MHz, CDCl<sub>3</sub>).

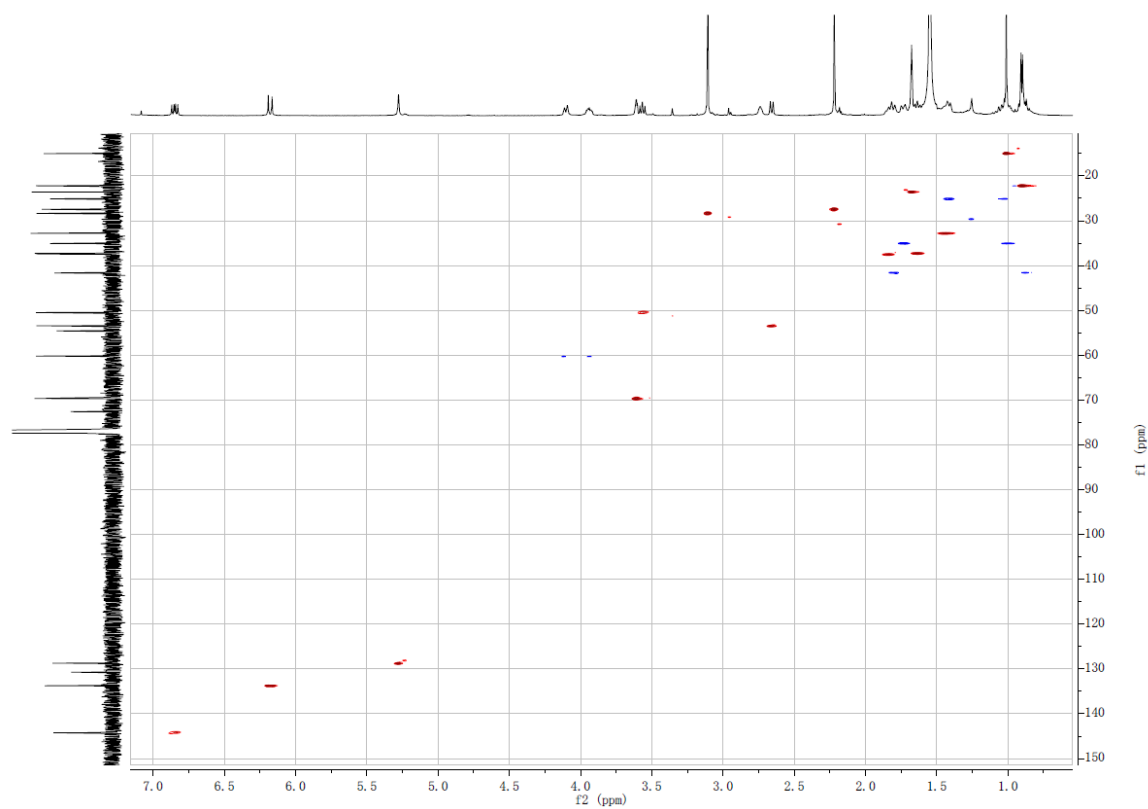


Figure S20. COSY spectrum of compound **3** (600 MHz, CDCl<sub>3</sub>).

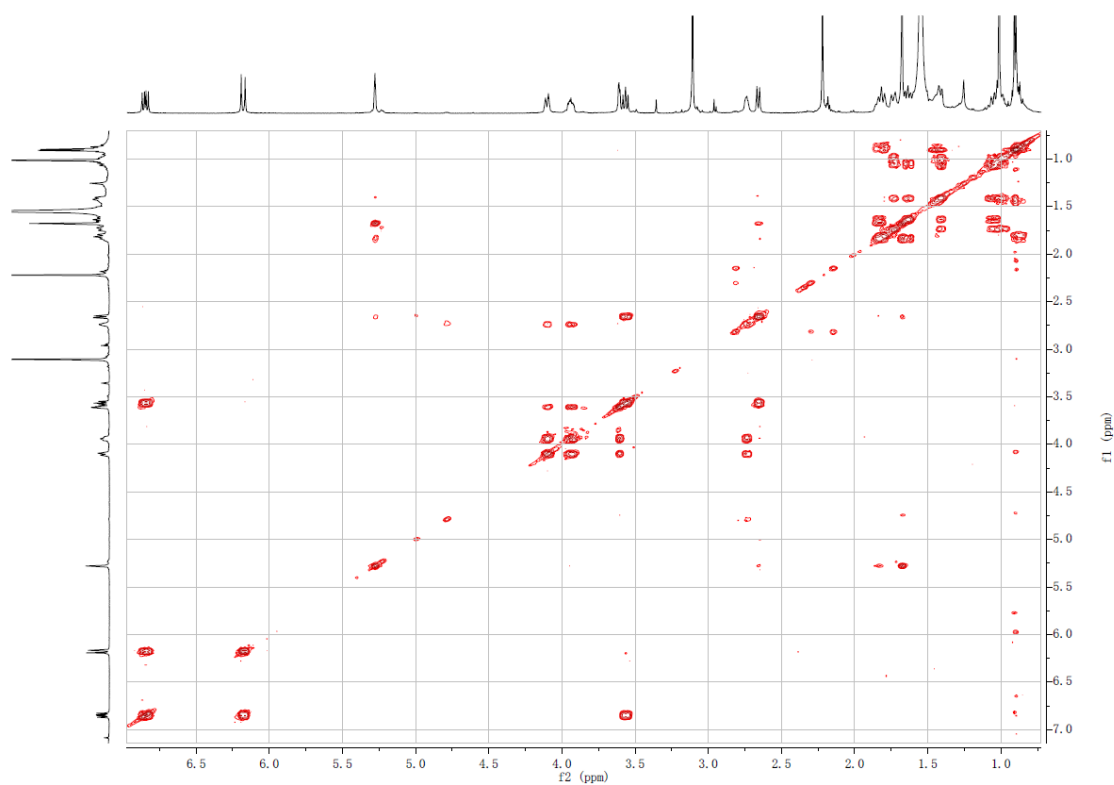


Figure S21. HMBC spectrum of compound 3 (600 MHz, CDCl<sub>3</sub>).

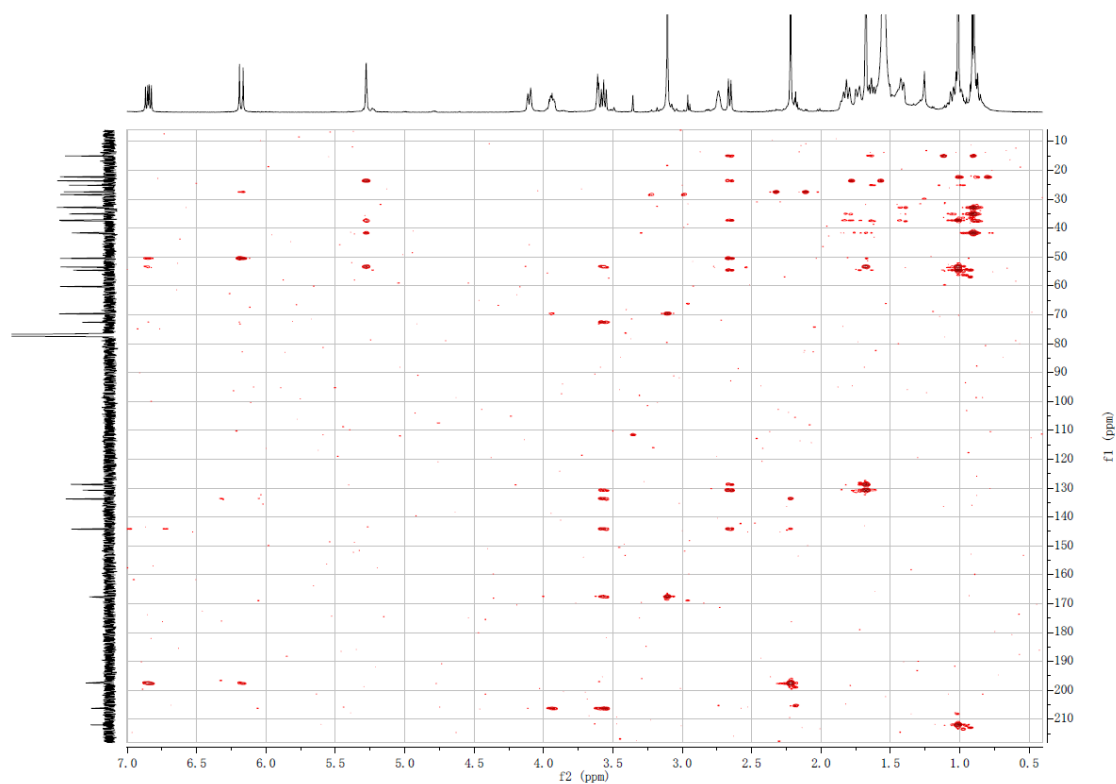
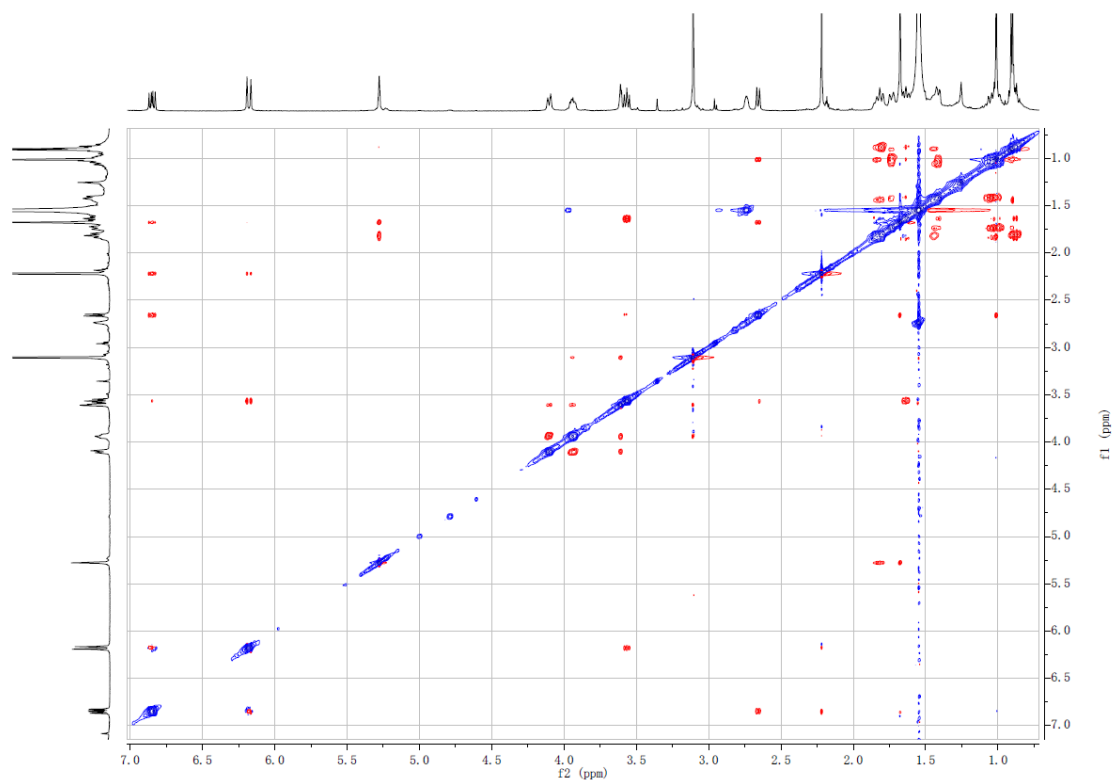
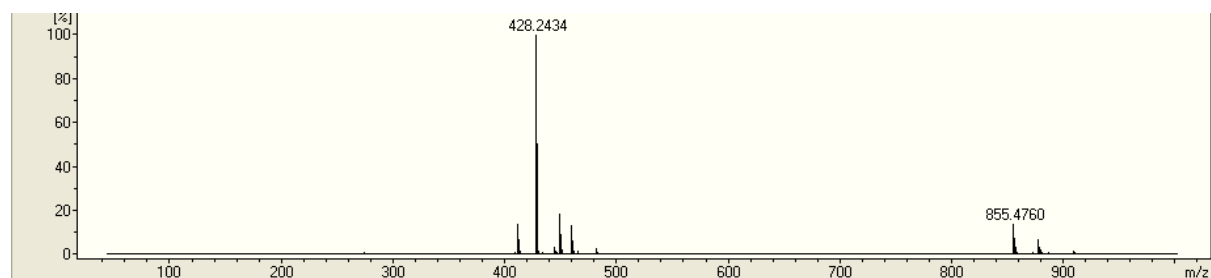


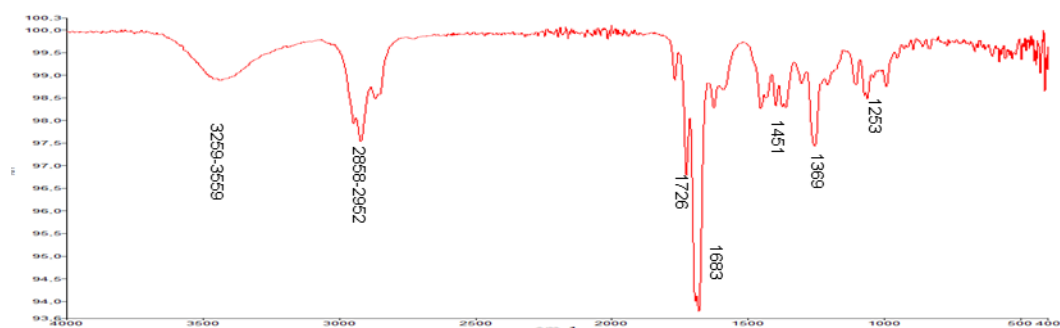
Figure S22. NOESY spectrum of compound 3 (600 MHz, CDCl<sub>3</sub>).



**Figure S23.** HR-ESIMS spectrum of compound **3**.

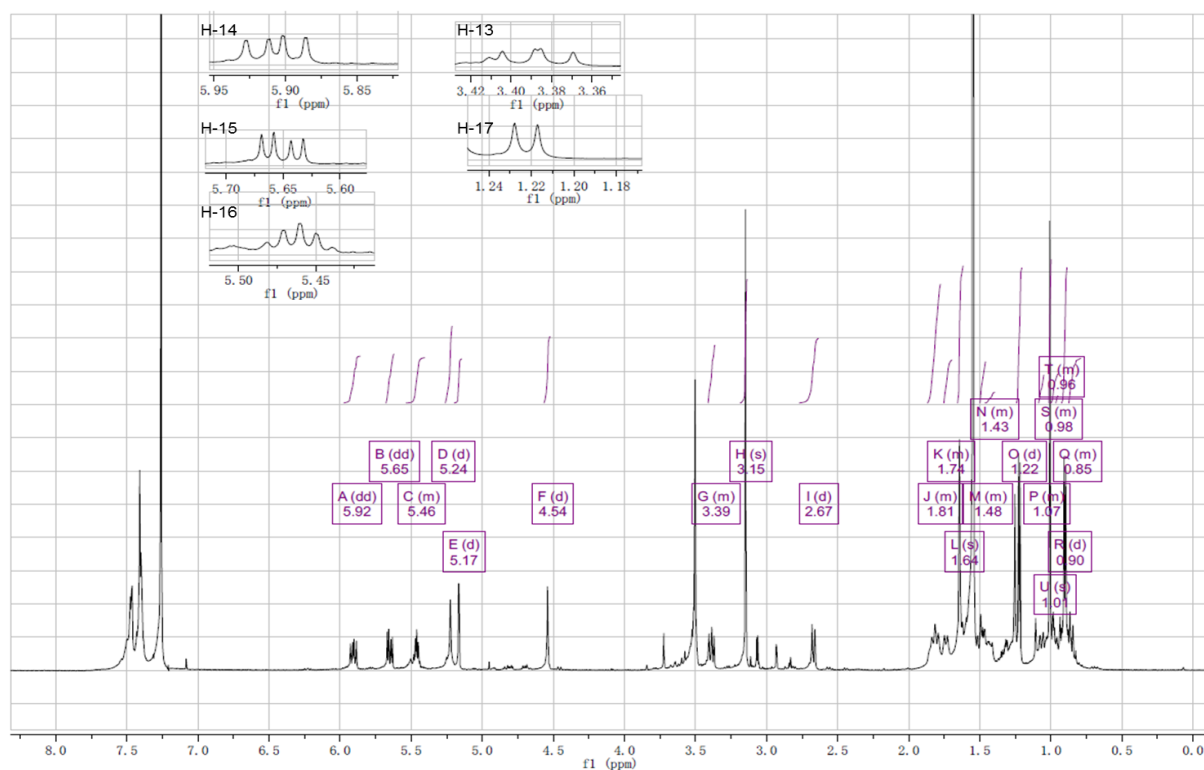


**Figure S24.** FT-IR spectrum of compound **3**.

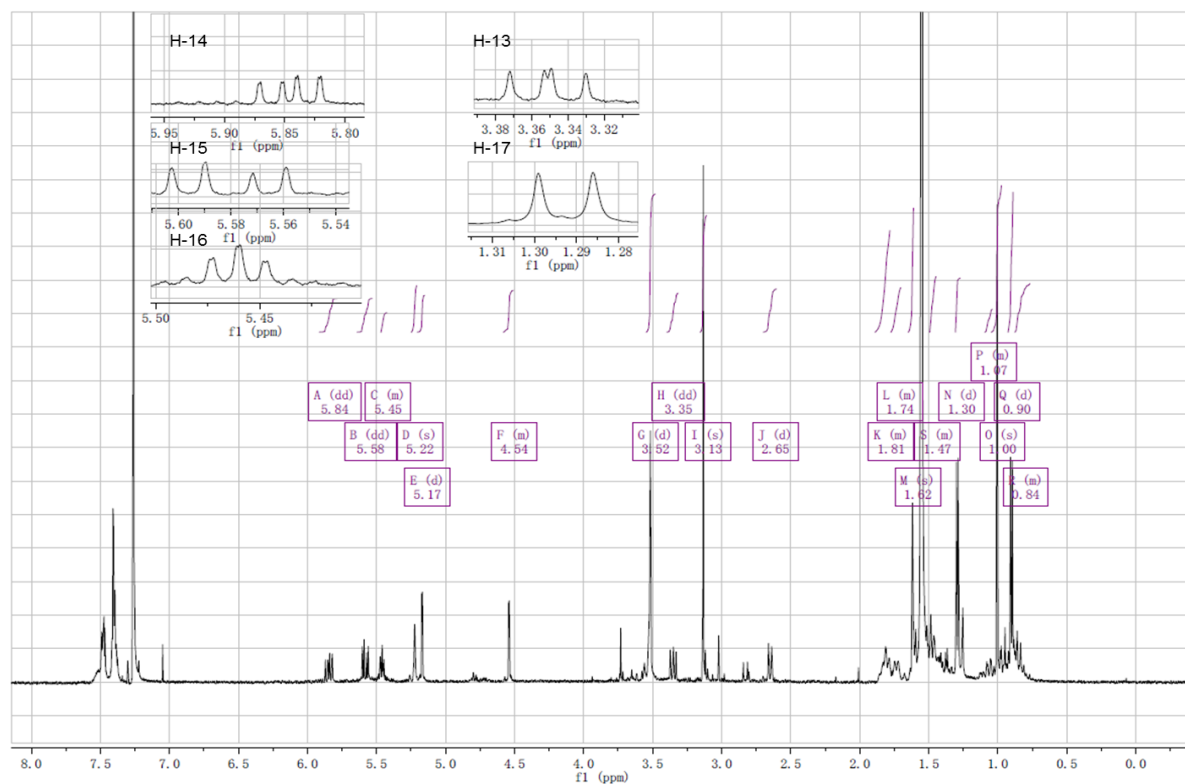




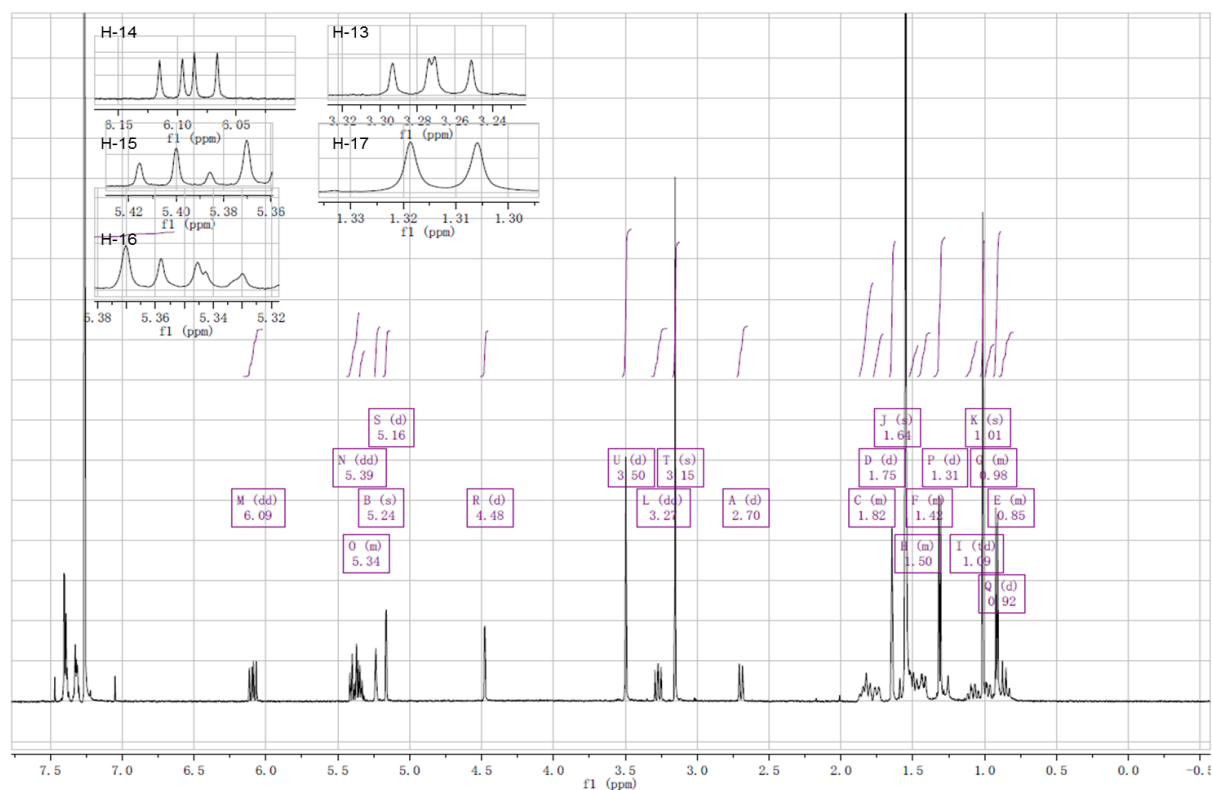
**Figure S25.**  $^1\text{H}$  NMR spectrum of 16-(*S*)-MTPA ester **6** (500 MHz,  $\text{CDCl}_3$ ).



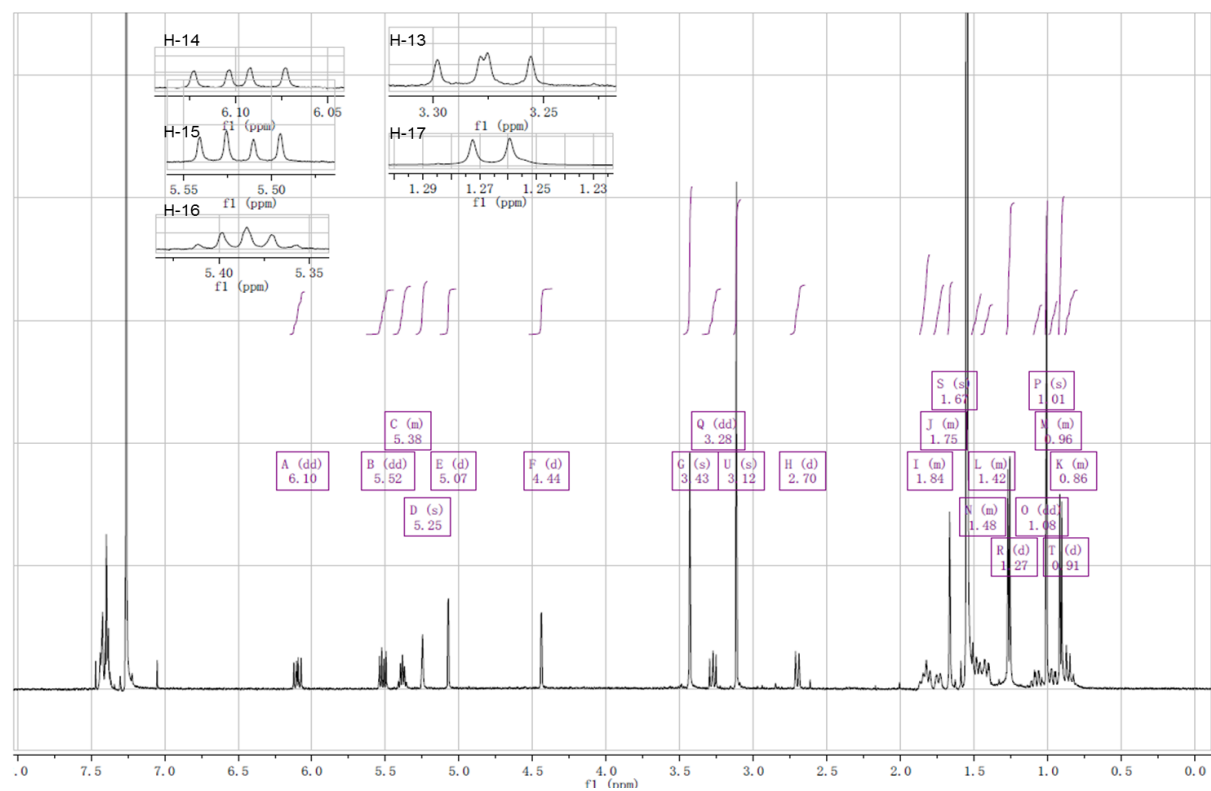
**Figure S26.**  $^1\text{H}$  NMR spectrum of 16-(*R*)-MTPA ester **7** (500 MHz,  $\text{CDCl}_3$ ).



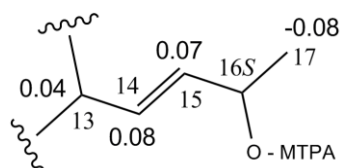
**Figure S27.**  $^1\text{H}$  NMR spectrum of 16-(*S*)-MTPA ester **8** (500 MHz,  $\text{CDCl}_3$ ).



**Figure S28.**  $^1\text{H}$  NMR spectrum of 16-(*R*)-MTPA ester **9** (500 MHz,  $\text{CDCl}_3$ ).



**Figure S29.**  $\Delta\delta(\delta_S\text{-}\delta_R)$  values (ppm) obtained from 16-MTPA esters (**6** and **7**) of compound **1**.



**Figure S30.**  $\Delta\delta(\delta_S\text{-}\delta_R)$  values (ppm) obtained from 16-MTPA esters (**8** and **9**) of compound **2**.

